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PHARMACOLOGICAL SPARING OF PROTEIN
IN BURN INJURY

ANNUAL REPORT

Robert R. Wolfe

Farook Jahoor

David N. Herndon

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<p>We have investigated the responsiveness of protein kinetics to insulin and the role of glucose oxidation rate as a mediator of the protein catabolic response to burn injury and sepsis by assessing the response of leucine and urea kinetics to a 5 hour hyperinsulinemic euglycemic clamp with and without the simultaneous administration of dichloroacetate DCA, (to further increase glucose oxidation via stimulation of pyruvate dehydrogenase activity) in 8 severely burned and 8 septic patients. Leucine and urea kinetics were measured by the primed-constant infusions of 1-¹³C-leucine and ¹⁵N₂-urea. Compared to controls, basal leucine kinetics (flux and oxidation) were significantly elevated (p < 0.01) in both groups of patients. Hyperinsulinemia elicited significant (p < 0.05) decreases in leucine kinetics in both groups of patients. Consistent with this observation, hyperinsulinemia caused urea production to decrease significantly (p < 0.05) in both patient groups. The administration of DCA to patients during hyperinsulinemia elicited a significant increase in glucose oxidation rate compared to the clamp rate (p < 0.05) and the percent of glucose</p>					
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uptake oxidized increased from $45.5 \pm 5.5\%$ to $53.5 \pm 4.8\%$; yet the response of leucine and urea kinetics to the clamp plus DCA was not different from the response to the clamp alone. These results suggest that the maximal effectiveness of insulin to suppress protein breakdown is not impaired and that a deficit in glucose oxidation or energy supply is probably not playing a major role in mediating the protein catabolic response to severe burn injury and sepsis.

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FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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INTRODUCTION:

Severe burn injury and sepsis are characterized metabolically by an increased resting energy expenditure, an elevated rate of lipolysis, persistent hyperglycemia (particularly when glucose is infused), and negative nitrogen balance (11, 14, 15, 31, 33, 35). Persistent protein loss presents an important clinical problem, since survival rate is inversely proportional to loss of lean body mass (8). However, vigorous nutritional support alone may often fail to entirely curtail protein wasting in severely catabolic patients (27). Furthermore, in such patients nutritional support may be without beneficial effects on immune function, hepatic function, or survival (10). Optimal metabolic support must therefore ultimately be directed at manipulation of the altered protein kinetics. If such manipulation is to be successful, it must be based on an understanding of the nature of the disturbance in protein metabolism.

Evidence indicates that resistance to the normal protein anabolic effect of insulin may be an important mechanism leading to net catabolism in severe injury or sepsis (9, 12, 23, 24). A general dysfunction of insulin is suggested by the failure of insulin to exert its normal hypoglycemic action (e.g., 34). It has been proposed that the failure of insulin to normally stimulate glucose uptake and oxidation could lead to protein catabolism indirectly, as a consequence of a peripheral energy deficit (23, 24). Another possible scenario is that because of the inability of insulin to restrain the stimulatory effect of glucagon on the rate of glucose production and gluconeogenesis, due to the increased plasma glucagon to insulin molar ratio (G/I ratio), there is an increased rate of protein breakdown to supply amino acids as substrates to fuel the accelerated rate of gluconeogenesis (3, 7). Alter-

natively, the recent work of Hasselgren, et al (9) indicates that in the skeletal muscle of septic rats there is an impairment of the direct action of insulin to inhibit protein breakdown and stimulate synthesis.

In contrast to these findings, other indirect lines of evidence suggest that the protein response to insulin may be reasonably intact. For example, we have previously reported that inhibition of endogenous insulin secretion in severely burned patients elicited a marked increase in alanine flux, indicative of an increased rate of peripheral nitrogen release, hence of protein breakdown, thereby suggesting that the ability of basal insulin to restrain protein breakdown was intact (14). This is supported by the in vitro findings of Odessey and Parr (18) who reported no impairment in the ability of physiological concentrations of insulin to stimulate protein synthesis and inhibit breakdown in the soleus muscle of burned rats.

In order to find out whether there is a defect in insulin's action on protein metabolism in severe burn injury and sepsis, in this study we have tested the maximum biological effectiveness of insulin on the absolute and net rates of protein breakdown using the hyperinsulinemic euglycemic clamp technique and infusions of stable isotopes of leucine and urea.

The maximal biological effectiveness of insulin was assessed because this approach is most likely to uncover a "post-receptor" defect in insulin action (22), and this has been postulated to be the basis of insulin resistance in severely burned patients (1). In order to assess more directly the possible role of a defect in glucose oxidation or energy deficit as a mediator of the net protein catabolic response we have evaluated in one group of patients the response of protein metabolism to the hyperinsulinemic clamp with and without

the simultaneous administration of dichloroacetate (DCA). DCA stimulates PDH activity and thus glucose oxidation.

METHODS:

Patients:

Eight severely burned and 8 septic patients who were admitted to the Burn, Surgical and Medical Intensive Care Units of The University of Texas Medical Branch in Galveston, Texas were studied. Characteristics of the individual patients are summarized in Tables 1a and 1b. The 7 male and 1 female burn patients ranged in age from 16 to 42 years and had a mean burn size of 65% of total body surface area (TBSA) of which 35% TBSA was third degree. At the time of study, between postburn days 4 to 17, all patients were in a hypermetabolic state as indicated by comparing their basal metabolic rate to expected values for normal subjects of similar age and size. They had a mean heart rate of 117 ± 4 beats per minute and systolic blood pressure of 126 ± 4 mmHg. Core temperature ranged from 37.3 to 39.0°C (Table 1a). Clinical treatment of all patients was comparable, with early excision of the burn wound and grafting as described in detail elsewhere (14).

Sepsis was assessed by the presence of infection in blood, cerebrospinal fluid, urinary tract, peritoneum, lung, wound or abscess, coupled with fever, leukocytosis, thrombocytopenia, altered mental status and hyperdynamic cardiovascular status (4, 26). The 1 female and 7 male septic patients ranged in age from 25 to 45 years (Table 1b). According to the classification of Elebute and Stoner (4) they had a mean sepsis score of 17 ± 1 at the time of study, and were in a hyperdynamic state with a mean cardiac index of 6.2 ± 0.3 $\text{l/m}^2/\text{min}$, heart rate of 112 ± 3 beats/min, systolic blood pressure of 127 ± 7 mmHg and core temperature ranging from 37.3 to 39.2°C .

Patients had no personal history of any metabolic diseases and at the time of study had normal renal function as judged by serum creatinine levels and urine output.

The experimental protocol was approved by the Institutional Review Board of The University of Texas Medical Branch and informed consent was obtained after the nature, purpose and potential risks of the study were explained in detail to the patient and/or relatives in the presence of a patient advocate.

Materials:

The isotopes infused as tracers were $1\text{-}^{13}\text{C}$ -leucine, 99% enriched, obtained from Tracer Technologies, Inc. (Somerville, MA) and $^{15}\text{N}_2$ -urea, 99.7% enriched, from Merck Isotopes (Montreal, Canada). Dichloroacetate (DCA) was obtained from CTC Organics (Atlanta, GA). Sterile solutions of the isotopes and DCA were prepared in 0.45% saline and passed through a 0.22 μm filter (Millipore Corp., Bedford, MA) into sterile evacuated containers. For each infusion an aliquot of the infusate was analyzed for the exact isotope concentration in order to calculate the actual infusion rate for each study. Insulin solution (2 U/ml) for infusion was prepared by adding regular human insulin (Novolin, E. R. Squibb & Sons, Princeton, N.J.) to normal saline containing 2.5 ml of the patient's blood. The isotopes and insulin solutions were infused using a Bard Model 2716 Syringe Infusion Pumps (Bard Med Systems, S. Natick, MA).

Experimental Design:

There were two different experimental protocols (Fig. 1), in both of which isotopes of leucine and urea were infused. The rate of appearance (R_a) of leucine was used as an index of the absolute rate of protein breakdown and its rate of oxidation as an index of net protein catabolism. Since all

studies were performed in fasted patients leucine being an essential amino acid could only have appeared from protein breakdown. Its flux can therefore be considered a good index of the absolute rate of protein. Secondly, because of its essentiality it cannot be re-synthesized after oxidation and is therefore unavailable to the body for protein synthesis. For this reason the rate of leucine oxidation can be considered as an index of net protein loss. Since urea synthesis represents the final pathway of nitrogen disposal, its rate of production was also used as a more direct indicator of net protein catabolism. The isotopic technique was used to quantify urea kinetics to overcome difficulty in accurately determining total urea excretion.

Protocol 1:

The primary aim of this protocol was to test the maximum biological effectiveness of insulin to restrain the absolute and net rates of protein breakdown in severely burned and in septic patients. After the bicarbonate pool was primed with 1.7 $\mu\text{mol/kg}$ of $\text{NaH}^{13}\text{CO}_3$, a primed-constant infusion of $^{15}\text{N}_2$ -urea (infusion rate of $0.14 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, prime 84 $\mu\text{mol/kg}$) and 1- ^{13}C -leucine (infusion rate of $0.25 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, prime 15 $\mu\text{mol/kg}$) was started and maintained throughout an 8 hour study period (Fig. 1). After 3 hours of isotope infusion only (basal period), the insulin pool was primed with a bolus dose, calculated to raise plasma concentration to 1000 $\mu\text{U/ml}$, followed by a constant infusion of insulin at the rate of $500 \text{ mU/m}^2 \text{ min}^{-1}$ for 5 hours. The insulin infusion was accompanied by a variable infusion of 20% D-glucose solution at a rate sufficient to maintain plasma glucose concentration at 90 mg/dl . During this hyperinsulinemic period plasma glucose level was closely monitored at 5 to 10 minute intervals using the glucose oxidase reaction

(glucose Analyzer 2, Beckman Instruments, Inc., Fullerton, CA). Serum potassium levels were monitored at 30 minute intervals and maintained within the normal range by infusing a 50 mEq/l KCl solution. The rate of delivery of maintenance fluids was appropriately adjusted during the study period in order to maintain a comparable fluid delivery rate and a constant state of hydration.

Blood and expired air samples were collected before the isotope infusions started and at 15-minute intervals during the last 90 minutes of the basal and hyperinsulinemic periods. During the course of a study the patients' metabolic rate (O_2 consumption, CO_2 production) was also measured in both periods, with a Horizon Metabolic Cart (Beckman Instruments, Inc., Fullerton, CA).

Protocol 2:

Eight patients (5 burned, 3 septic, Tables 1a and 1b), who had already participated in protocol 1, were studied for a second time in this protocol. The aim of this protocol was to determine the relationship between the rate of glucose oxidation and leucine and urea kinetics during the hyperinsulinemic clamp. This was accomplished by studying each patient twice. The first study was as described for protocol 1. The same protocol was then repeated, only at the start of the hyperinsulinemic period and then 2.5 hours later, DCA was infused at a rate sufficient to deliver a dose of 35 mg/kg over 30 min. DCA was given in order to stimulate pyruvate dehydrogenase activity, and thereby increasing the fraction of pyruvate entering the TCA cycle for ultimate oxidation.

Analysis of Samples:

Blood samples were collected in ice-cold heparinized tubes and stored on ice until the end of the experiment, when plasma was separated by centrifuga-

tion at 4°C. Aliquots of plasma were placed in tubes containing EDTA and trasylol and stored at -20°C for insulin determinations. For the determination of amino acid concentrations, aliquots of plasma were immediately deproteinized with ice-cold 15% sulfosalicylic acid and stored at -20°C for analysis the following day. The rest of plasma was stored at -20°C to be analyzed later for the isotopic enrichments of urea and alpha-ketoisocaproic acid (alpha-KICA), the ketoacid analog of leucine.

Expired air was collected in 3-liter anesthesia bags and CO₂ immediately trapped as NaHCO₃ by bubbling through 0.1 M NaOH solution and stored in sealed containers.

Amino acid concentrations were determined by column chromatography on a model 121M autoanalyzer (Beckman Instruments, Inc., Fullerton, CA), and insulin was determined with an RIA kit (Instar, Stillwater, MN). The isotopic enrichments of plasma urea and alpha-KICA were determined with a Hewlett-Packard 5985B GCMS system (Palo Alto, CA). Urea enrichment was measured by GCMS analysis of its N, N¹-bistrimethylsilyl derivative during electron impact ionization and monitoring of ions at m/e 189 and 191. The enrichment of alpha-KICA was measured on its silylquinoxalinol derivative using electron impact ionization and monitoring ions at m/e 232.2 and 233.2 (25).

The enrichment of carbon dioxide was measured using a dual inlet triple collector isotope ratio mass spectrometer (Nuclide, State college, PA). The gas was released from the NaHCO₃ solution by reacting with H₃PO₄ in vacuo and introducing directly into the inlet system of the mass spectrometer.

Calculations:

In all the experiments an isotopic steady state was achieved for alpha-KICA and urea permitting the use of a standard steady state equation to

calculate urea production rate and the whole-body flux of leucine.

$$Ra = \left(\frac{IE_{\text{Infusion}}}{IE_{\text{Plasma}}} - 1 \right) \times F$$

where Ra (umol/kg·min) is the rate of production of urea or whole-body flux of leucine, IE is isotopic enrichment in mole percent excess and F = rate of infusion of the isotope (umol·kg⁻¹·min⁻¹). Plasma alpha-KICA enrichment was used to calculate whole-body leucine flux because leucine is approximately in isotopic equilibrium with its transamination product alpha-KICA intracellularly (17) before the latter is released into plasma. Since intracellular enrichment of an amino acid is optimal for calculating its total rate of release as a consequence of protein breakdown, plasma alpha-KICA enrichment more accurately reflects the true intracellular enrichment of leucine than does plasma leucine enrichment (17).

Whole-body leucine oxidation rate was calculated from the following equation:

$$\text{Leucine Oxidation} = \frac{IE_{\text{CO}_2} \times \dot{V}\text{CO}_2 / 0.81}{IE_{\text{alpha-KICA}}}$$

where IE_{CO_2} is the enrichment of expired CO₂, $IE_{\text{alpha-KICA}}$ is the enrichment of plasma alpha-KICA and $\dot{V}\text{CO}_2$ is the rate of production of CO₂ in umol·kg⁻¹·min⁻¹. To account for bicarbonate retained in the body pool the rate of excretion of ¹³CO₂ is divided by 0.81 (25). Plasma alpha-KICA enrichment is used as the precursor pool enrichment because all alpha-KICA is derived from

leucine intracellularly. Therefore plasma alpha-KICA enrichment reflects the true enrichment of the leucine precursor pool from which decarboxylation takes place.

During the hyperinsulinemic clamp the preferential oxidation of infused glucose over other fuel substrates will lead to an increased rate of excretion of $^{13}\text{CO}_2$ because of the relatively higher naturally occurring ^{13}C content of the infused dextrose (13, 14). The rate of excretion of $^{13}\text{CO}_2$ during the hyperinsulinemic clamp, therefore had to be corrected in order to account for the fraction of $^{13}\text{CO}_2$ originating from the increased rate of glucose oxidation (25). This was accomplished by using the relationship between the rate of uptake of infused glucose and background $^{13}\text{CO}_2$ enrichment we determined separately in euglycemic clamp experiments in which no specifically enriched isotope was infused.

Glucose oxidation was calculated from the rate of utilization of O_2 , and the rate of excretion of CO_2 and N_2 as described by Frayn, et al, (5). N excretion rate was deduced from the isotopically determined rate of urea production assuming that the amount of N used for urea synthesis was a good approximation of the total rate of N loss from the body. This assumption is based on the fact that urea Ra measured isotopically over-estimates actual urea excretion rate by about 20% and urea excretion rate on the other hand only represents about 80% of total N excretion.

Statistical Analysis:

Dunnett's (the paired) t-test was used to compare basal data with data obtained during the hyperinsulinemic period (protocol 1) or the hyperinsulinemic plus DCA period (protocol 2). To compare data from burned patients with data from septic patients the non-paired t-test was used. The paired t-test

was also used for the comparison of the results during the clamp and with those during clamp plus DCA (protocol 2). Results are presented as mean \pm SEM.

RESULTS:

Protocol 1:

Values for the normal postabsorptive state were obtained from a control study performed concurrently in healthy normal volunteers (25).

In the burned and septic patients the insulin infusion caused comparable increases in plasma insulin levels from within the normal range (9.6 ± 1.9 and 8.6 ± 1.1 uU/ml respectively) to 1298 ± 347 and 1213 ± 229 uU/ml respectively. Mean basal glucose concentration was identical in both groups, 108 ± 6 mg/dl, and during the insulin infusion was clamped at exactly 91 ± 1.1 and 91 ± 0.7 mg/dl respectively in each group. This required a glucose infusion rate of 11.5 ± 0.7 mg \cdot kg $^{-1}\cdot$ min $^{-1}$ in burned patients and 6.5 ± 1.2 mg \cdot kg $^{-1}\cdot$ min $^{-1}$ in septic patients, (significantly different, $p < 0.01$).

In burned and septic patients basal leucine Ra was 5.15 ± 0.24 and 4.08 ± 0.22 umol \cdot kg $^{-1}\cdot$ min $^{-1}$ respectively (Table 2) about 2 and 1.5 times the control value of 2.78 ± 0.16 umol \cdot kg $^{-1}\cdot$ min $^{-1}$, indicating a significant stimulation of the absolute rate of protein breakdown in both groups of patients. Nonetheless, the hyperinsulinemic clamp reduced Ra leucine in both patient groups to a comparable extent as that in controls (Table 2). However, whereas in absolute terms the decrease in leucine Ra elicited by hyperinsulinemia in the septic patients (1.09 ± 0.09 umol \cdot kg $^{-1}\cdot$ min $^{-1}$) (Figure 2) was comparable to that of the controls (1.15 ± 0.12 umol \cdot kg $^{-1}\cdot$ min $^{-1}$) and even greater ($p < 0.01$) in the burns, (1.76 ± 0.16 umol \cdot kg $^{-1}\cdot$ min $^{-1}$), the insulin infusion still failed to reduce leucine Ra to a normal level in either group, (3.39 ± 0.14 in burns,

2.99 \pm 0.19 in sepsis versus 1.64 \pm 0.08 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in controls, Table 2).

The basal rates of leucine oxidation in the burned, (1.6 \pm 0.14 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and septic patients (1.25 \pm 0.15 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), were more than 2 times the control rate of 0.61 \pm 0.07 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (Table 2). As in the case of Ra leucine, the insulin infusion significantly reduced ($p < 0.05$) leucine oxidation in both groups of patients (Table 2) by amounts comparable to the decrease in the control group (from 0.61 \pm 0.01 to 0.32 \pm 0.01 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). As with leucine flux, however, the insulin infusion failed to reduce leucine oxidation in either patient group to the value for controls (0.32 \pm 0.03 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Compared to control values (16), basal urea Ra was elevated by about 100% and 50% in the burn and septic patients respectively (Table 2). Consistent with the effect of insulin on reducing leucine flux and oxidation, the insulin infusion elicited significant and comparable reductions ($p < 0.05$) in urea Ra in both groups of patients (Table 2).

In both groups of patients the insulin infusion elicited significant reductions ($p < 0.05$, < 0.01) in the concentrations of all plasma amino acids (Table 3).

Protocol 2:

The insulin infusion alone caused plasma insulin levels to increase from a basal value of 9.6 \pm 1.9 $\mu\text{U}/\text{ml}$ to 1132 \pm 143 $\mu\text{U}/\text{ml}$. The simultaneous administration of DCA did not affect this response as basal insulin increased from 9 \pm 1.1 $\mu\text{U}/\text{ml}$ to 1165 \pm 196 $\mu\text{U}/\text{ml}$. Mean basal glucose concentration was 90 \pm 4 and 100 \pm 7 mg/dl , respectively, during the two studies. During the insulin infusion, glucose was clamped at 90 \pm 1.3 mg/dl and when DCA was also infused it was clamped at about the same level, 91 \pm 0.5 mg/dl . This required a

glucose infusion rate of 9.7 ± 1.2 mg/kg·min in the hyperinsulinemic clamp alone (protocol 1) and 10.1 ± 1.1 mg/kg·min when DCA was also given (protocol 2). Since endogenous glucose production is totally suppressed during the hyperinsulinemic clamp period (26), the rate of glucose infusion is equal to the rate of glucose uptake, suggesting that DCA administration had no additive effect on the rate of glucose uptake during the hyperinsulinemic clamp. In response to the clamp (\pm DCA) the rate of CO_2 production and the respiratory quotient (RQ) increased significantly in both studies (Table 4). Basal RQ increased significantly from 0.80 ± 0.02 to 0.92 ± 0.02 during the clamp without DCA ($p < 0.01$), and the additional administration of DCA caused basal RQ to increase even further from 0.78 ± 0.01 to 0.97 ± 0.02 ($p < 0.01$), confirming the stimulatory action of DCA on pyruvate dehydrogenase. This is shown in Figure 3 where hyperinsulinemia and hyperinsulinemia plus DCA causes the basal rate of glucose oxidation to increase significantly ($p < 0.01$) from 1.61 ± 0.24 to 4.60 ± 0.62 mg kg^{-1} min^{-1} and from 1.15 ± 0.30 to 5.70 ± 1.0 mg kg^{-1} min^{-1} respectively. DCA increased the percent of glucose uptake oxidized during the clamp from $45.5 \pm 5.5\%$ to $53.5 \pm 4.8\%$ ($p < 0.05$). In absolute terms the increase in glucose oxidation rate when DCA was also given, 4.5 ± 0.73 mg kg^{-1} min^{-1} was significantly greater ($p < 0.05$) than the increase observed during the clamp alone, 2.98 ± 0.65 mg kg^{-1} min^{-1} (Figure 3). Preferential oxidation of pyruvate during DCA treatment is also suggested by the fact that there were greater decreases ($p < 0.01$) in both plasma lactate and alanine concentrations during clamp plus DCA than clamp alone (Table 5).

Both the hyperinsulinemic clamp and the clamp plus DCA elicited significant and comparable reductions in leucine Ra from a basal value of 4.59 ± 0.34 to 3.05 ± 0.19 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and from 4.55 ± 0.34 to 2.54 ± 0.27 $\mu\text{mol} \cdot \text{kg}^{-1}$.

min⁻¹ respectively, (Table 6). In absolute terms although the reduction in leucine Ra due to the clamp plus DCA (decrease of 2.01 ± 0.34 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was 30% greater, it was not significantly different ($p = 0.076$) from the reduction of 1.54 ± 0.18 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ due to the clamp alone. Six out of 8 patients, however, responded with a greater decrease in leucine Ra (due to the DCA) which was significantly different ($p < 0.05$) from the decrease in leucine Ra elicited by the clamp alone. Similarly the rates of leucine oxidation and urea production were suppressed comparably by insulin, regardless of the presence of DCA (Table 6).

As in protocol 1, the hyperinsulinemic clamp elicited significant reductions in the plasma concentrations of all amino acids (Table 7). DCA did not markedly affect this response, although the decreases in aspartic acid, glycine and histidine, failed to reach a level of significance when DCA was given, and the decrease in alanine concentration, 159 ± 13 $\mu\text{mol}/\text{ml}$, was twice the magnitude of the decrease 79 ± 8 $\mu\text{mol}/\text{ml}$ ($p < 0.01$) due to the clamp alone (Table 5).

DISCUSSION:

The primary aim of this study was to find out whether there was a defect in insulin's action on protein metabolism in burn injury and sepsis and the role of glucose oxidation in mediating the protein catabolic response in these patients. We found that hyperinsulinemia reduced leucine flux and rate of oxidation to the same extent as in normal controls but failed to normalize either. These results suggest that there is no impairment in the maximum biological effectiveness of insulin to inhibit the absolute and net rates of protein breakdown in severe burn injury and sepsis. However, the failure of hyperinsulinemia to normalize leucine kinetics, means that a possible role of

insulin resistance in mediating the elevated basal rate of protein catabolism cannot be ruled out in these patients. Secondly, since the increase in glucose oxidation during the hyperinsulinemic clamp failed to normalize leucine and urea kinetics in both groups of patients, and since the further stimulation of the rate of glucose oxidation by DCA administration failed to significantly affect either leucine or urea kinetics, it can be concluded that the protein catabolic response in these patients was not mediated via a defect in glucose oxidation or a deficit in peripheral energy supply secondary to an impairment in glucose oxidation. Finally, since endogenous glucose production was totally suppressed during the hyperinsulinemic clamp (26), the failure to normalize leucine and urea kinetics during the clamp in these studies suggests that an increased utilization of amino acids to fuel an accelerated rate of gluconeogenesis is not the major cause of the stimulated rate of net protein catabolism in burn injury and sepsis.

The present finding that the absolute and net rates of protein breakdown are markedly stimulated in severe burn injury and sepsis is in agreement with our previous reports (15, 34) and those of others (3, 7, 11). The suppression of leucine flux and oxidation by hyperinsulinemia has been reported by us and other investigators both in normal volunteers (25, 29) and in severely traumatized patients (2), suggesting that the protein sparing effect of insulin demonstrated in severely traumatized patients by Allison and others (11) is due to the ability of insulin to restrain both the absolute and net rates of protein breakdown. Since the extent to which hyperinsulinemia inhibited the absolute and net rates of protein breakdown in each group of patients in this study was comparable to its effect in normal volunteers (25), it can be concluded that there is no impairment in the maximum biological effectiveness of

insulin to restrain absolute and net protein catabolism in burn injury and sepsis. These results are in agreement with the in vivo findings of, Inculet, et al (12) in post-operative trauma patients, and the in vitro findings of Odessey and Parr (18) in the soleus muscle of burned rat and Ryan, et al (24) in the skeletal muscle of septic rabbit, that insulin administration suppresses net protein breakdown but fails to reduce it to control or pre-operative levels. In the only other study employing the hyperinsulinemic euglycemic clamp to investigate the role of insulin in mediating the protein catabolic response to trauma, Brooks, et al (2) reported a four-fold suppression of amino nitrogen efflux from the skeletal muscle bed of injured patients to the level seen in normal control subjects. This response of amino-N efflux to the hyperinsulinemic clamp was, however, far more pronounced than that observed in our study or in the study of Inculet, et al (12). Furthermore, both our present study and that of Inculet, et al (12) failed to demonstrate a normalization of leucine or amino-N flux, or leucine oxidation in response to the insulin infusion. On the other hand, only the in vitro study of Hasselgren, et al (9) reported a failure of hyperinsulinemia (1000 uU/ml) to elicit any suppression of the elevated rate of muscle (EDL) protein breakdown in septic rats.

The normal responsiveness of protein kinetics to hyperinsulinemia in severe burn injury and sepsis as demonstrated in this study does not rule out a possible role of insulin resistance in mediating the elevated basal protein catabolism in these patients. The fact that the same degree of hyperinsulinemia failed to reduce the absolute and net rates of protein catabolism in both groups of patients to the extent seen in normal volunteers, supports the contention of Inculet, et al (12) that insulin resistance or factors acting

via insulin resistance may be responsible for mediating the net protein catabolic response to injury and sepsis. Alternatively it could be argued that the ability of insulin to reduce but not normalize the absolute and net rates of protein breakdown could be due to some of the proteins whose breakdown is markedly accelerated not being responsive to insulin under any condition.

Our results also fail to support any possible role of an impairment in glucose oxidation and/or a deficit in peripheral energy supply in mediating the stimulated rates of absolute and net protein catabolism in severe burn injury and in sepsis. The concept that there is an increase in muscle protein breakdown to supply amino acids as substrates in order to correct a deficit in peripheral energy availability arose out of the combined in vitro work of Ryan, et al (23, 24) and in vivo findings of O'Donnell, et al (19, 20). Based on their findings that septic rats had a markedly reduced blood FFA concentration, a reduced rate of uptake and oxidation of glucose by muscle (due to an inhibition of muscle PDH activity) coupled with an increased rate of leucine oxidation and decreased rate of muscle protein synthesis, Ryan, et al (23, 24) proposed that in sepsis there is a reduced mobilization of fat without a concomitant increase in glucose oxidation in muscle, resulting in an inadequate supply of metabolic fuel for muscle. This in turn promotes amino acid oxidation by muscle to meet cellular energy requirements (23, 24). This proposal received further in vivo support from the work of O'Donnell, et al in the septic pig (19) and in septic patients (20) and by Clowes, et al in severely traumatized and septic patients (3). However, our results show that although there is an impairment in the ability of septic (but not burned) patients to take up glucose (26), once the glucose has entered the cell there is no defect

in the ability of burned or septic patients to oxidize glucose (Ref. 26 and Figure 3) as the percent of glucose uptake oxidized is the same (40%) in burns, sepsis and controls in both the basal state and during the clamp (26). Also, in the past we have shown that the basal rate of uptake of glucose and the rate of lipolysis in severely burned and septic patients are markedly elevated compared to control values (14, 31, 34). Thus in spite of the fact that during the hyperinsulinemic clamp the increase in the amount of glucose oxidized in the burn patients, $3.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, was more than twice the increase in septic patients, $1.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (26) the reduction in the flux and rate of oxidation of leucine in both groups of patients were comparable (Table 2), suggesting that the reduction in the absolute and net rates of protein breakdown during the hyperinsulinemic clamp was probably due to the direct protein sparing action of insulin and not to an increase in energy availability resulting from the stimulated rate of glucose oxidation. Secondly, since it can be calculated from our indirect calorimetry data (Table 4) that the rate of fat oxidation remains unchanged when DCA is administered during the hyperinsulinemic clamp, the further increase in glucose oxidation, of $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in response to the DCA treatment (Figure 3) should have spared an equivalent amount of amino acid from being oxidized. This should translate into a decrease in the rate of urea production. In reality, however, the additional stimulation of glucose oxidation by DCA did not elicit any additional decrease in the rate of production of urea (Table 6).

Differences in experimental approaches may explain why studies such as those of O'Donnell, et al (19, 20) and Clowes, et al (3) have reported a deficit in peripheral energy supply in sepsis, whereas our studies employing stable isotope tracer techniques have consistently indicated the absence of an

impairment in glucose oxidation (14, 34) and an increased rate of lipolysis and fat oxidation in severely burned or septic patients (31, 32), sufficient to meet the stimulated basal energy requirements of severe trauma (33). A potential flaw of studies estimating energy availability by measuring substrate balance across an organ or tissue bed, as was done in the earlier studies (3, 19, 20), is that such net balance techniques do not take into account substrates such as glycogen and FFA which can be released within the tissue bed or organ and oxidized for energy without being released into the blood compartment. Hence A-V balance measurements will always underestimate the actual amount of substrates available to the organ or tissue bed for oxidation.

It should be mentioned that the further decrease in leucine Ra elicited by the addition of DCA, although not statistically significant, was 30% greater than the decrease elicited by the clamp alone (Table 6), suggesting that the increased glucose oxidation (or energy supply) elicited a further reduction in the absolute rate of protein breakdown. However, because the change in leucine oxidation rate elicited by both the clamp and the clamp plus DCA was identical (Table 6) this merely means that the non-oxidative disposal rate of leucine (or protein synthesis) was also suppressed to a greater extent when the DCA was given. That is, in terms of net protein catabolism the decrease was the same in both situations. However, protein turnover (breakdown plus synthesis) rate was suppressed when DCA was also administered.

Another point that warrants discussion is the fact that in vitro studies have shown that DCA by itself suppresses leucine oxidation in muscle preparations (21, 28) and elicits an increased release of BCAA's from the perfused hindquarter of fasted rats (6). Translated to the situation in vivo in the

whole-body, one would therefore predict that a direct DCA effect on leucine kinetics will cause a further decrease in leucine oxidation (adding to the decrease elicited by hyperinsulinemia alone) and a lesser decrease in leucine flux compared to the decrease elicited by the clamp alone. The fact that neither of these observations were made rules out any direct DCA effect on leucine kinetics. It should also be pointed out that the dose of DCA used to elicit a response in leucine metabolism in both the in vitro studies (21, 28) and the perfused rat hindquarters study (6) was six times the dose given in the present study.

Lastly, our results do not support the role of an accelerated rate of gluconeogenesis as a major mediator of the increased net protein catabolism in severe burn injury and sepsis, as proposed by some investigators (3, 7). The increased glucagon/insulin molar ratio in stressed patients increases hepatic glucose output (30), in part by stimulating the rate of gluconeogenesis (7). It has been argued that this, in turn, causes an increase in peripheral protein breakdown in order to supply amino acids as substrates to fuel this accelerated rate of gluconeogenesis (7). Our results, however, show that during the hyperinsulinemic clamp endogenous glucose production is totally suppressed (26), suggesting that hepatic gluconeogenesis is also totally suppressed; yet the absolute and net rates of protein breakdown are still elevated and fail to reach a normal control value. This would suggest that the accelerated rate of gluconeogenesis is not solely responsible for the increased absolute rate of protein breakdown. On the other hand, accelerated irreversible deamination of amino acid precursors for gluconeogenesis presumably plays a role in the high net rate of protein breakdown in severely burned and septic patients.

The important clinical finding in this study is that the stimulated rate of net protein catabolism in severely burned and septic patients is not mediated via a defect in glucose metabolism or a deficit in energy supply. Hence therapy (such as the provision of excessive quantities of glucose plus insulin, e.g., ref. 11 or stimulating glucose oxidation w. DCA), designed to reduce protein loss by stimulating glucose uptake and oxidation will have a minimal sparing effect only. It is therefore important that the potential deleterious side-effects that can result from such therapy, for example, chronic hyperglycemia and fatty infiltration of the liver (10), be carefully weighed against its protein anabolic benefits when it is being considered for treatment.

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TABLE 1a - CHARACTERISTICS OF BURN PATIENTS

Patient	Age Yr.	Sex	Weight (kg)	Percent BSA Burn Total/3	Day	Postburn Day Studied Protocol #	Heart Rate (beats/min)	Blood Pressure (mm Hg)	Temper- ature °C
A*	31	M	64	47/4	14;17	1;2	130;116	128;126	38.7;38
B*	20	M	71	51/12	13;16	1;2	104;95	132;135	38.6;38.6
C*	16	M	65	75/33	8;11	1;2	130;122	130;145	38.1;37.7
D*	50	M	83	48/5	12;15	1;2	114;131	110;100	38;39
E*	42	M	86	45/15	5;8	1;2	95;104	141;108	37.7;38.6
F	21	M	61	83/80	13	1 125	120	37.3	
G	18	F	45	72/60	4	1 122	133	37.8	
H	28	M	90	81/60	16	1 136	132	37.7	

TABLE 1b - CHARACTERISTICS OF SEPTIC PATIENTS

Patient	Age	Sex	Weight (kg)	Diagnosis	Sepsis Score	Cardiac Index (l/min/m ²)	Heart Rate (beats/min)	Blood Pressure (mm Hg)	Temper- ature °C
I	45	M	85	Perforated Appendicitis	14	7.2	102	160	37.8
J*	33	M	125	Small Bowel Infarcation	18	5.3;5.5	108;112	116;114	37.6;38
K	35	M	62	90% BSA Burn	17	5.9	129	129	38.3
L	28	M	99	50% BSA Burn	12	8.8	114	126	37.3
M*	41	M	90	Pneumonia	20	5.5;5.4	128;96	121;100	37.9;37.3
N	35	M	71	Pancreatic Abscess	16	6.8	123	169	37.7
O	44	F	57	Pneumonia	22	6.2	118	100	38.9
P*	43	M	81	43% BSA burn; Pancreatitis	16	5.9;5.9	101;105	132;130	39.2;38.5

* Patient studied in protocols 1 and 2.

TABLE 2
RESPONSE OF LEUCINE AND UREA KINETICS TO HYPERINSULINEMIC
EUGLYCEMIC CLAMP

Subject Group	Basal	Clamp
	<u>Leucine Ra (umol·kg⁻¹·min⁻¹)</u>	
Control * (n=6)	2.78 ± 0.16	1.64 ± 0.08 ⁺
Burn (n = 8)	5.15 ± 0.24	3.39 ± 0.14 ⁺
Sepsis (n = 8)	4.08 ± 0.22	2.99 ± 0.19 ⁺⁺
	<u>Leucine Oxidation</u>	
Control *	0.61 ± 0.07	0.32 ± 0.01 ⁺
Burn	1.60 ± 0.14	1.00 ± 0.11 ⁺
Sepsis	1.25 ± 0.15	1.06 ± 0.11 ⁺⁺
	<u>Urea Ra</u>	
Control (n = 14) **	4.20 ± 0.37	---
Burn	8.19 ± 0.92	7.01 ± 0.66 ⁺⁺
Sepsis	6.23 ± 1.15	5.49 ± 0.99 ⁺⁺

Values are means ± SEM.

+, ++, Significantly different from basal value (p < 0.01, p < 0.05) respectively.

* From Reference No. 24.

** From Reference No. 16.

TABLE 3

RESPONSE OF PLASMA AMINO ACID CONCENTRATIONS TO A HYPERINSULINEMIC
EUGLYCEMIC CLAMP IN BURN AND SEPTIC PATIENTS (Values are Mean + SEM)

Amino Acid	Burn		p*	Sepsis		p
	Basal	Clamp		Basal	Clamp	
	μM			μM		
Aspartate	24 \pm 2	16 \pm 1	< 0.01	26 \pm 3	21 \pm 3	< 0.01
Glutamate plus Glutamine	296 \pm 32	202 \pm 20	< 0.01	410 \pm 41	262 \pm 33	< 0.01
Alanine	193 \pm 21	130 \pm 15	< 0.01	280 \pm 42	205 \pm 29	< 0.01
Glycine	141 \pm 10	110 \pm 4	< 0.01	177 \pm 19	144 \pm 16	< 0.01
Serine	79 \pm 7	43 \pm 4	< 0.01	76 \pm 4	54 \pm 5	< 0.01
Proline	144 \pm 15	85 \pm 8	< 0.01	227 \pm 49	149 \pm 29	< 0.01
Threonine	79 \pm 7	38 \pm 4	< 0.01	89 \pm 8	56 \pm 6	< 0.01
Methionine	25 \pm 2	10 \pm 1	< 0.01	35 \pm 6	17 \pm 3	< 0.01
Lysine	148 \pm 6	96 \pm 4	< 0.01	172 \pm 19	124 \pm 13	< 0.01
Histidine	56 \pm 4	45 \pm 3	< 0.05	96 \pm 17	78 \pm 14	< 0.01
Valine	191 \pm 21	75 \pm 9	< 0.01	179 \pm 23	103 \pm 22	< 0.01
Isoleucine	60 \pm 9	21 \pm 3	< 0.01	58 \pm 4	21 \pm 4	< 0.01
Leucine	127 \pm 13	60 \pm 6	< 0.01	144 \pm 19	78 \pm 14	< 0.01
Tyrosine	61 \pm 5	29 \pm 2	< 0.01	70 \pm 5	44 \pm 4	< 0.01
Phenylalanine	78 \pm 4	54 \pm 3	< 0.01	115 \pm 5	86 \pm 6	< 0.01
Arginine	69 \pm 6	45 \pm 6	< 0.01	79 \pm 7	54 \pm 6	< 0.01

*Significance of difference from basal.

TABLE 4
EFFECT OF DICHLOROACETATE ON THE METABOLIC RESPONSE TO THE
HYPERINSULINEMIC EUGLYCEMIC CLAMP (PROTOCOL 2)
(Values are Mean \pm SEM)

Metabolic Parameter	Basal	Clamp	Basal	Clamp + DCA
$\dot{V}O_2$ (ml/min)	415 \pm 29	439 \pm 17	415 \pm 35	421 \pm 24
$\dot{V}CO_2$ (ml/min)	331 \pm 17	407 \pm 19 ⁺	322 \pm 24	408 \pm 25 ⁺
RQ*	0.80 \pm 0.02	0.92 \pm 0.02 ⁺	0.78 \pm 0.01	0.97 \pm 0.02 ⁺
REE* (kcal/m ² /d)	1388 \pm 103	1487 \pm 89	1412 \pm 106	1534 \pm 55

*RQ is Respiratory Quotient.

REE is resting energy expenditure.

⁺Significantly different from basal value (p < 0.01).

TABLE 5

EFFECT OF DICHLOROACETATE ON PLASMA LACTATE, ALANINE AND UREACONCENTRATIONS DURING HYPERINSULINEMIC EUGLYCEMIC CLAMP(Values are mean \pm SEM)

<u>Substrate</u>	<u>Basal</u>	<u>Clamp</u>	<u>Basal</u>	<u>Clamp + DCA</u>
Lactate (umol/ml)	2.31 \pm 0.79	3.09 \pm 0.91 ⁺	2.03 \pm 0.33	0.91 \pm 0.08 ⁺
Alanine (umol/ml)	250 \pm 36	171 \pm 23 ⁺	230 \pm 26	71 \pm 6 ⁺
Urea (umol/ml)	7.69 \pm 1.52	6.02 \pm 1.05 ⁺	6.32 \pm 0.80	4.97 \pm 0.68 ⁺

⁺ Significantly different from basal value, (p < 0.05).

TABLE 6
EFFECT OF DICHLOROACETATE ON RESPONSE OF LEUCINE AND UREA KINETICS
TO HYPERINSULINEMIC EUGLYCEMIC CLAMP (PROTOCOL 2)

	Basal	Clamp	Basal	Clamp + DCA
	(umol • kg ⁻¹ • min ⁻¹)			
Leucine Ra	4.59±0.34	3.05±0.19 ⁺	4.55±0.34	2.54±0.27 ⁺
△ Leucine Ra		1.54±0.18		2.01±0.24
Leucine Oxidation	1.58±0.16	0.94±0.11 ⁺	1.40±0.13	0.79±0.03 ⁺
△ Leucine Oxidation		0.64±0.13		0.62±0.09
Urea Ra	7.86±1.09	6.67±0.8 ⁺⁺	6.53±0.61	5.78±0.05 ⁺⁺
△ Urea Ra		1.19±0.27		0.75±0.12

Values are mean ± SE for 8 patients (5 burns, 3 septic).

*, **, Significantly different from basal value (p < 0.01, p < 0.05), respectively.

△ Change from basal due to the clamp (Basal-Clamp) and due to clamp + DCA (Basal - Clamp + DCA).

TABLE 7
EFFECT OF DICHLOROACETATE ON THE RESPONSE OF PLASMA AMINO ACID
CONCENTRATIONS TO THE HYPERINSULINEMIC EUGLYCEMIC (PROTOCOL 2)
Values are Mean \pm SEM

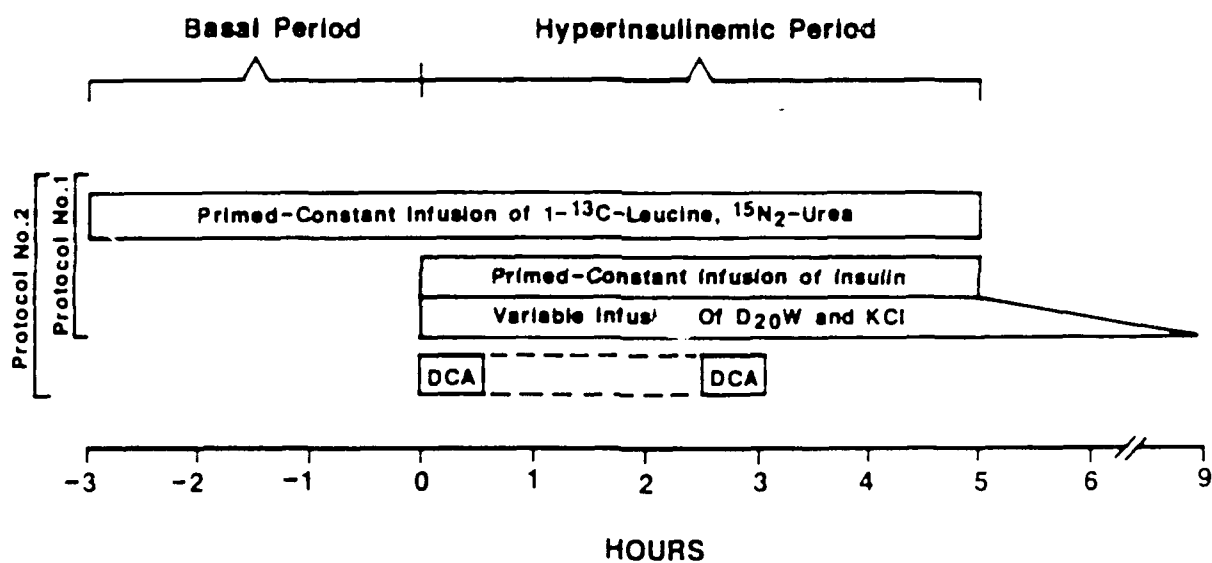
Amino Acid	Basal μM	Clamp	p	Basal μM	Clamp + DCA	p
Aspartate	26 \pm 1	18 \pm 2	0.01	22 \pm 2	18 \pm 2	N.S.
Glutamate plus Glutamine	370 \pm 30	216 \pm 21	< 0.01	318 \pm 44	210 \pm 10	< 0.01
Alanine	250 \pm 36	171 \pm 23	< 0.01	231 \pm 26	71 \pm 6	< 0.01
Glycine	161 \pm 17	125 \pm 13	< 0.01	152 \pm 12	130 \pm 5	N.S.
Serine	84 \pm 5	47 \pm 3	< 0.01	75 \pm 6	42 \pm 3	< 0.01
Proline	193 \pm 45	115 \pm 28	< 0.01	170 \pm 16	76 \pm 9	< 0.01
Threonine	91 \pm 7	46 \pm 6	< 0.01	87 \pm 7	45 \pm 6	< 0.01
Methionine	31 \pm 5	14 \pm 3	< 0.01	24 \pm 1	9 \pm 1	< 0.01
Lysine	158 \pm 10	105 \pm 9	< 0.01	162 \pm 16	108 \pm 7	< 0.01
Histidine	76 \pm 16	60 \pm 13	< 0.01	60 \pm 6	50 \pm 9	N.S.
Valine	205 \pm 22	88 \pm 12	< 0.01	187 \pm 27	85 \pm 13	< 0.01
Isoleucine	66 \pm 7	20 \pm 2	< 0.01	60 \pm 6	22 \pm 3	< 0.01
Leucine	149 \pm 14	71 \pm 10	< 0.01	131 \pm 16	60 \pm 6	< 0.01
Tyrosine	71 \pm 3	35 \pm 4	< 0.01	69 \pm 7	35 \pm 5	< 0.01
Phenylalanine	92 \pm 6	62 \pm 7	< 0.01	83 \pm 9	53 \pm 5	< 0.01
Arginine	78 \pm 5	55 \pm 3	< 0.01	76 \pm 6	50 \pm 4	< 0.05

*Significance of difference from basal values (paired t-test).

FIGURE LEGENDS

- Figure 1: A schematic representation of the experimental protocols.
- Figure 2: Decrease from basal value of leucine Ra and oxidation due to the hyperinsulinemic clamp in protocol 1. C = control subjects; B = burn patients, S = septic patients. Results are mean \pm SEM.
+Significantly different from control value ($p < 0.01$).
- Figure 3: Response of glucose oxidation rate to the hyperinsulinemic clamp (\pm DCA) in protocol 2. Values are mean \pm SEM.
 Δ indicates increase from basal value.
*Significantly different from basal value ($p < 0.01$).
+Significantly different from hyperinsulinemic period ($p < 0.05$).

Experimental Protocols



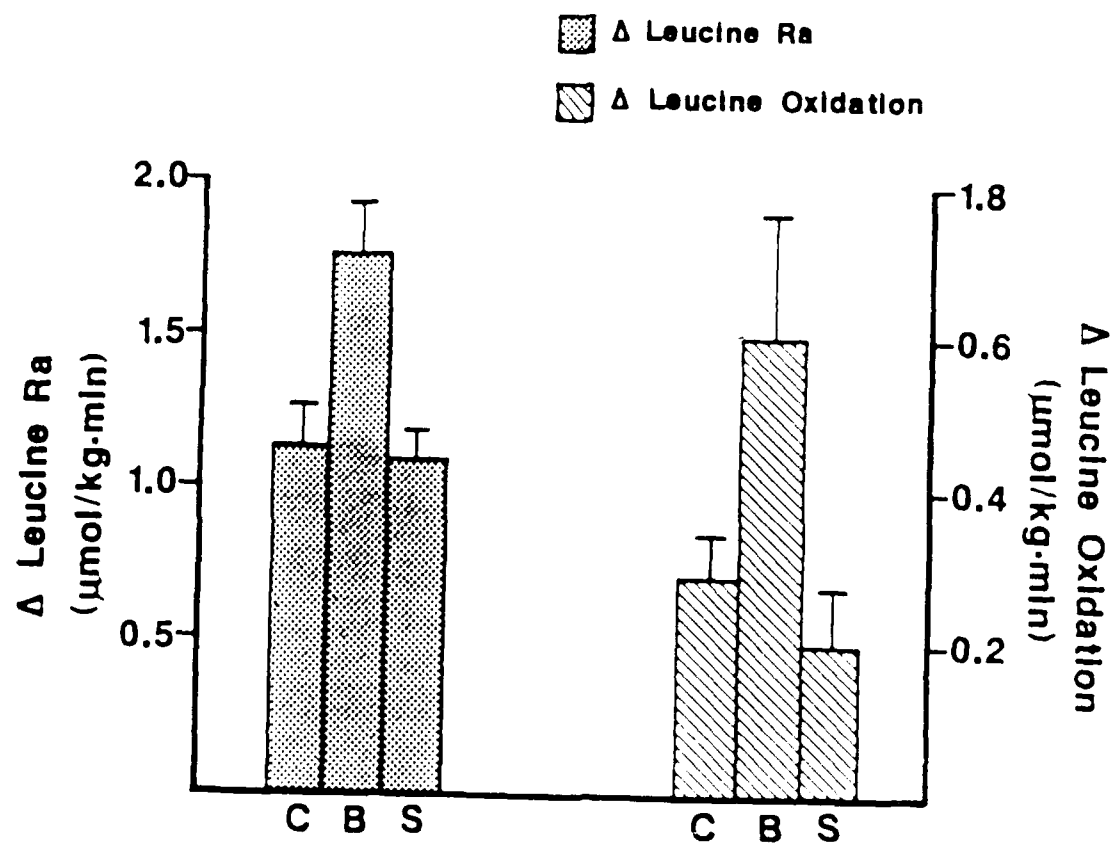


Fig. 3

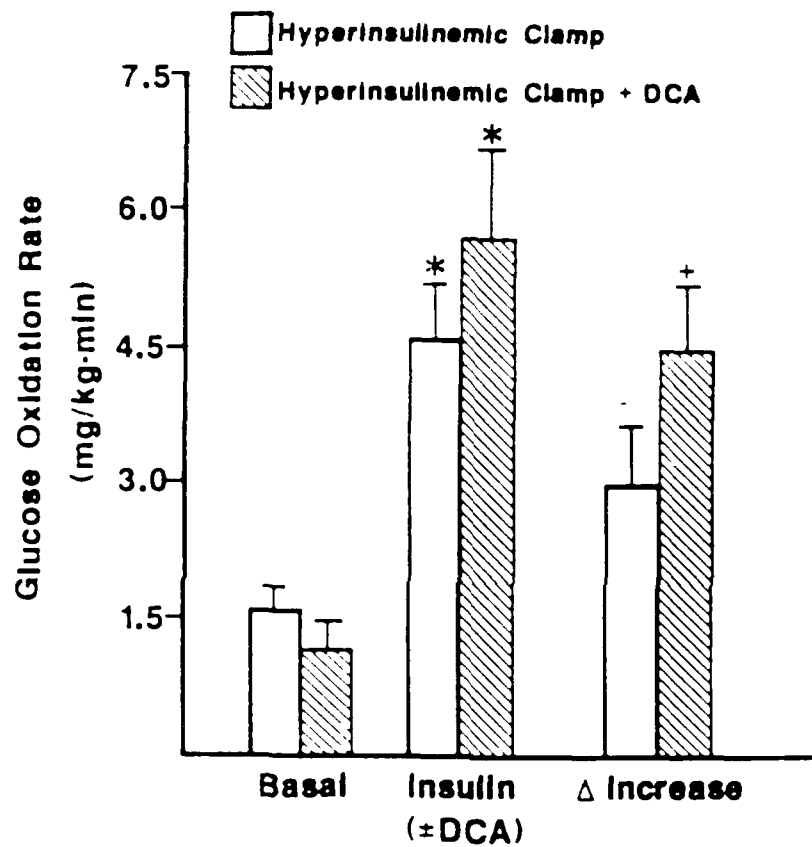


Fig. 3